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(54) Title: THE CYTOPLASMIC INHIBITION OF GENE EXPRESSION

(57) Abstract

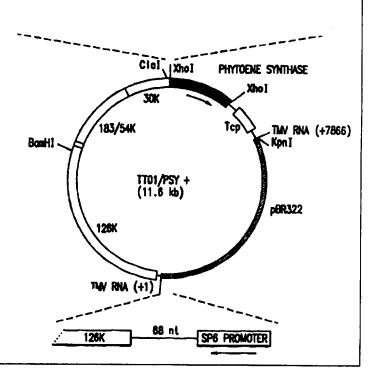
One aspect of the invention is to provide novel genetic constructions for the expression of inhibitory RNA in the cytoplasm of eukaryotic cells. The inhibitory RNA may be an anti-sense RNA or a co-suppressor RNA. The genetic constructions of the invention are capable of replicating in the cytoplasm of a eukaryotic cell and comprise a promoter region in functional combination with an encoding polynucleotide. The genetic constructions may be designed so as to replicate in the cytoplasm of plant cells, yeast cells, and mammalian cells. When the eukaryotic cell of interest is a plant cell, the genetic construction is preferably derived from a plant RNA virus. Plant RNA virus derived genetic constructions may employ a plant virus subgenomic promoter, including subgenomic promoters from tobamoviruses in functional combination with the RNA encoding region. Another aspect of the invention is to provide cells comprising the genetic constructions of the invention and organism comprising a plurality of such cells. Another aspect of the invention is to provide methods of reducing the expression of a gene of interest in eukaryotic cells, i.e., methods of producing eukaryotic cells exhibiting reduced levels of expression of a gene of interest. The methods of the invention comprise the step of transfecting a cell with a genetic construction of the invention in which the RNA encoding region is specific for the gene of interest. Another aspect of the invention is to provide plant cells that produce elevated levels of the carotenoid phytoene. The elevated levels of phytoene are achieved by inhibiting the expression at the enzyme phytoene desaturase using the vectors of the invention.

tsp Xhel Start codon
GTTI[AAATAGGCTGGGGTTTAAAAT ATG TGT GTT GCT GTT GTT GCT TGT GACMet Ser Vol Ale Leu Leu Try Vol Vol Ser Pro Cys Asp

TRANSIT PEPTIDE OF PRITORIE STRITHASE

GTC TCA AAT GGG ACA AGT TTC ANG GAA TCA GTC GGG GAG GGA AAC GGT

Well Ser Ann Gly The Ser Phe Het Glu Ser Vel Arg Glu Gly Asn Arg



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INTERNATIONAL SEARCH REPORT

Inte onal Application No PCT/US 95/06741

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/83 C12N1 A01H5/00

C12N15/11

C12N15/53

C12N15/52

C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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03.01.96

"&" document member of the same patent family

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20 December 1995

Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 95/06741

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INTERNATIONAL SEARCH REPORT

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Inte onal Application No
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